AMENDMENT TO THE CLAIMS

Please enter the following amendments to the claims without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows:

1. (currently amended three times) A method of producing a red blood cell comprising an agent the method comprising: a first step of pre-sensitizing a red blood cell *in vitro* or *ex vivo*, followed by a second step of loading said red blood cell with an agent, wherein the first step of pre-sensitizing and the second step of loading are temporally separated.

2. (previously amended) A method according to claim 1, wherein said second step comprises loading said red blood cell with a first agent and a second agent.

3. (currently amended) A method according to claim 1, further comprising the step of electrosensitising the cell *in vitro* or *ex vivo*.

4. (currently amended three times) A method for selectively releasing an agent from a red blood cell comprising the steps of:

(a) pre-sensitising a red blood cell *in vitro* or *ex vivo*;

(b) loading said red blood cell with an agent;

(c) electrosensitising said red blood cell *in vitro* or *ex vivo*; and

(d) effectuating substantial release of said agent from said sensitised red blood cell by applying ultrasound at a frequency and energy sufficient to cause disruption of sensitised red blood cells,

wherein the first step of pre-sensitising and the second step of loading are temporally separated.

5-9 (cancelled)

10. (previously amended) A method according to claim 1, wherein said pre-sensitising step comprises applying an electric field to said red blood cell.

11. (previously twice amended) A method according to claim 1, wherein said pre-sensitising step further comprises applying ultrasound to the red blood cell.

12. (previously amended) A method according to claim 1, wherein said loading step comprises hypotonic dialysis.

13. (original) A method according to claim 3, wherein said electrosensitising step comprises applying an electric field to said red blood cell.

14. (original) A method according to claim 13, wherein said electric field applied to said red blood cell ranges from 0.1 kV/cm to 10 kV/cm.

15. (original) A method according to claim 13, wherein said electric field is applied to said red blood cell 1 microsecond to 100 milliseconds.

16. (original) A method according to claim 3, wherein said electrosensitisation step is performed after said loading step.

17. (original) A method according to claim 3, wherein said electrosensitisation step is performed before said loading step.

18. (original) A method according to claim 4, wherein said ultrasound is selected from the group consisting of diagnostic ultrasound, therapeutic ultrasound and a combination of diagnostic and therapeutic ultrasound.

19. (original) A method according to claim 4 wherein the applied ultrasound energy source is at a power level from about 0.05 W/cm^2 to about 100 W/cm^2.

20-25 (cancelled)

26. (currently amended) A ~~red blood cell~~ composition comprising an electrosensitised red blood cell hypersensitive to a disruptive stimulus obtainable by a method comprising:

(a) presensitising a red blood cell *in vitro* or *ex vivo*;

(b) loading the cell with an agent, wherein the amount of agent that is loaded into a presensitised red blood cell is higher that the amount loaded into a red blood cell that is not presensitised; and

(c) electrosensitising the cell *in vitro* or *ex vivo*;

thereby resulting in an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised.

27-29 (cancelled)

30. (currently twice amended) A pharmaceutical composition comprising ~~a~~ an electrosensitised red blood cell ~~composition~~ hypersensitive to a disruptive stimulus made by a process comprising:

(a) pre-sensitizing a red blood cell *in vitro* or *ex vivo*; ~~and~~

(b) loading said red blood cell with an agent, wherein the amount of agent that is loaded into a presensitised red blood cell is higher that the amount loaded into a red blood cell that is not presensitised; and

(c) electrosensitising the cell *in vitro* or *ex vivo*,

thereby resulting in an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised.

31. (currently amended three times) The composition of claim 30 wherein ~~said~~ the electrosensitised red blood cell ~~composition further comprises a red blood cell~~ is immunocompatible in a vertebrate.

32. (currently twice amended) The composition of claim 31 wherein the electrosensitised red blood cell comprises polyethylene glycol (PEG).

33-35 (cancelled)

36. (previously added) The method of claim 3, wherein the further step of electrosensitizing the cell is performed after loading.

37. (previously added) The method of claim 3, wherein the further step of electrosensitizing the cell is performed before loading.

38. (previously added) The method of claim 4, wherein the electrosensitization comprises the step of applying an electric field to said cell, said electric field being in the form of multiple electrical pulses.

39. (previously added) The method of claim 38, wherein said pulses are delivered as a wave form selected from the group consisting of an exponential wave form, a square wave form and a modulated wave form.

40. (previously added) The method of claim 4, wherein said loading is performed by osmotically shocking said cell.

41. (previously added) The method of claim 4, wherein multiple different agents are loaded into the cell.

42. (previously added) The method of claim 4, wherein after loading, said cells are re-sealed.

43. (previously added) A method according to claim 18, wherein the diagnostic ultrasound energy source is at a power level of up to about 100 W/cm^2.

44. (previously added) A method according to claim 18, wherein the diagnostic ultrasound energy source is at a power level of up to about 750 W/cm^2.

45. (previously added) A method according to claim 18, wherein the therapeutic ultrasound energy source is at a power level of 3-4 W/cm^2.

46. (previously added) A method according to claim 18, wherein the therapeutic ultrasound energy source is at a power level of up to about 100 W/cm^2.

47. (previously added) A method according to claim 18, wherein the therapeutic ultrasound energy source is at a power level of up to about 1 kW/cm^2.

48. (previously added) A method according to claim 1, further comprising a third step of electrosensitizing the red blood cell, the third step being carried out before or after the second step of loading said red blood cell with an agent.

49. (cancelled)

Please cancel claim 49 without pre without prejudice, without admission, without surrender of subject matter and without intention of creating any estoppel as to equivalents.

REMARKS

Reconsideration and withdrawal of the rejections of this application and consideration and entry of this paper are respectfully requested in view of the herein remarks and accompanying information, which place the application in condition for allowance.

1. Status of Claims and Formal Matters

Claims 1-4, 10-19, 26, 30-32 and 36-49 are under consideration in this application. Claims 1, 3, 4, 26 and 30-32 have been amended and claim 49 has been cancelled. No new matter has been added by this amendment.

Support for the recitation of temporal separation of the first step of pre-sensitizing and the second step of loading are temporally separated in claims 1 and 4 is found on page 11, line 4 of the specification. Support for the recitation of *in vitro* or *ex vivo* electrosensitisation in claim 3 and 26 is found on page 4, lines 10-12 and page 16, lines 22-23. Support for the recitation of an electrosensitised red blood cell hypersensitive to a disruptive stimulus relative to unelectrosensitised cells in claim 26 and 30 is found on page 11, lines 28-29, page 12, lines 9-10, and page 38, lines 11-15. Support for the recitation of the amount of agent that is loaded into a presensitised red blood cell is higher that the amount loaded into a red blood cell that is not presensitised is found on page 3, lines 21-22. Support for the recitation of electrosensitising the cell *in vitro* or *ex vivo* in claim 30 is from cancelled claim 49. The recitation of an electrosensitised red blood cell in claim 31 and 32 is for sufficient antecedent basis, i.e., the cell of claims 31 and 32 refers to the electrosensitised red blood cell of claim 30.

The Examiner is thanked for withdrawing the provisional rejection of claims 4, 18, and 19 under 35 U.S.C. § 102(e) and the rejection of claims 4, 18, and 19 under 35 U.S.C. § 102(f). The Examiner is thanked for indicated that claims 11 and 12 would be allowable if rewritten in independent form. Claims 1, 3, 26 and 30-32 have been amended without prejudice, without admission, and without surrender of subject matter, and without any intention of creating any estoppel as to equivalents to recite the base claim and intervening claims.

It is submitted that the claims, herewith and as originally presented, are patentably distinct over the prior art cited by the Examiner, and that these claims were in full compliance with the requirements of 35 U.S.C. § 112. The amendments of the claims, as presented herein,

are not made for purposes of patentability within the meaning of 35 U.S.C. §§§§ 101, 102, 103 or 112. Rather, these amendments and additions are made simply for clarification and to round out the scope of protection to which Applicants are entitled.

2. The Rejections Under 35 U.S.C. § 112, First Paragraph, Are Overcome

Claim 26 was rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement for presensitizing the red blood cell with electroporation *in vivo*. This claim has been amended to clarify that sensitizing a red blood cell occurs *in vitro* or *ex vivo*.

It is believed that the rejection under 35 U.S.C. § 112, first paragraph, has been overcome. Reconsideration and withdrawal are requested.

3. The Rejection Under 35 U.S.C. § 112, Second Paragraph, Is Overcome

Claim 49 was rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for insufficient antecedent basis. This claim has been cancelled, rendering the rejection moot.

It is believed that the rejection under 35 U.S.C. § 112, second paragraph, has been overcome. Reconsideration and withdrawal are requested.

4. The Rejections Under 35 U.S.C. § 102 Are Overcome

Claims 1, 3, 10, 14-17, 26, 30, 31, 36, 37, and 49 are rejected under 35 U.S.C. § 102(f) over U.S. Application No. 09/779,186, now U.S. Patent 6,495,351, because the response allegedly has not made clear whether the applications are commonly owned at the time of Applicants' invention.

In response, Applicants respectfully point out that U.S. Application No. 09/779,186, now U.S. Patent 6,495,351, is a continuation-in-part of the present invention, U.S. Application No. 09/748,789. Since U.S. Application No. 09/779,186, now U.S. Patent 6,495,351, is a continuation-in-part of the present application, both claim benefit to the same earlier filed application, i.e., U.S. Provisional Application No. 60/181,796, filed on February 11, 2000, and United Kingdom Application No. 002856.3, filed February 8, 2000. In addition, the present application and U.S. Application No. 09/779,186, now U.S. Patent 6,495,351, are both assigned

to Gendel, Ltd., and the claimed subject matter of both applications were invented by persons who, at the time of the invention, were obligated to assign to Gendel, Ltd. Furthermore, the Assignment of the present invention, recorded February 20, 2001 on Reel 011536 Frame 0628 states that the "undersigned hereby agree, upon the request and at the expense of said assignee, its successors and assigns, to execute any and all divisional, continuation and substitute applications for said invention or improvements, and any necessary oath, affidavit or declaration relating thereto". Thus, the subject matter in both the present application and U.S. Application No. 09/779,186, now U.S. Patent 6,495,351, was, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person. Thus, the rejection under 35 U.S.C. § 102(f) is precluded since the present invention is entitled to the benefit of an earlier filed application and both the present invention and U.S. Application No. 09/779,186, now U.S. Patent 6,495,351, are owned by the same entity at the time of the invention. See Manual of Patent Examining Procedure ("MPEP") § 2137.01.

Claims 26, 30, 31 and 49 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Mouneimne *et al.*, U.S. Patent No. 5,236,835 (hereinafter "Mouneimne"). This rejection is traversed.

Claims 26, 30, 31 and 49 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Lizano *et al.*, Biochimica Biophysica Acta 1998;1425:328-336 (hereinafter "Lizano"). This rejection is traversed.

Claims 26, 30, 31 and 49 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Mitchell *et al.*, Biotech Applied Biochem 1990;12:264-75 (hereinafter "Mitchell"). This rejection is traversed.

Claims 26, 30, 31 and 49 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Zimmerman *et al.*, U.S. Patent No. 4,289,756 (hereinafter "Zimmerman"). This rejection is traversed.

Claims 26, 30-32 and 49 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Magnani *et al.*, U.S. Patent No. 6,139,836 (hereinafter "Magnani"). This rejection is traversed.

It is respectfully pointed out that a two-prong inquiry must be satisfied in order for a Section 102 rejection to stand. First, the prior art reference must contain all of the elements of

the claimed invention. *See Lewmar Marine Inc. v. Barient Inc.,* 3 U.S.P.Q.2d 1766 (Fed. Cir. 1987). Second, the prior art must contain an enabling disclosure. *See Chester v. Miller,* 15 U.S.P.Q.2d 1333, 1336 (Fed. Cir. 1990). A reference contains an enabling disclosure if a person of ordinary skill in the art could have combined the description of the invention in the prior art reference with his own knowledge of the art to have placed himself in possession of the invention. *See In re Donohue,* 226, U.S.P.Q. 619, 621 (Fed. Cir. 1985).

Applying the law to the instant facts, the references relied upon by the Office Action does not disclose, suggest or enable Applicants' invention. At the outset, Applicants respectfully point out that claim 49 has been cancelled, thereby obviating the rejection to claim 49. Claims 26 and 30 relate to compositions comprising an electrosensitised red blood cell that is loaded with a higher amount of agent as compared to a non-presensitised cell and hypersensitive to a disruptive stimulus as compared to a non-electrosensitised cell. Claims 31 and 32 depend from claim 30. The red blood cells of Mouneimne, Lizano, Mitchell, Zimmerman, or Magnani do not disclose, suggest or enable the electrosensitised red blood cells of the present invention.

The electrosensitised red blood cells of the present invention are not merely red blood cells loaded with agents by electroporation as generally performed in the art. Rather, the electrosensitised red blood cells have efficient loading characteristics and are more susceptible to disruption by exposure to a stimulus. Applicants found that if red blood cells are subject to a dialysis loading step, the sensitivity of the loaded cells to ultrasound is reduced (*see e.g.,* page 3, lines 7-8 of the specification). The Applicants determined that this reduction to sensitivity can be reversed by subjecting the cells to an additional sensitisation step, red blood cells can be produced that have excellent loading characteristics and ultrasound sensitivity so as to allow highly efficient unloading of agents at low exposures of ultrasound (*see e.g.*, page 3, lines 10-13). Thus, the present invention relates to an improved method for both improved loading and selective release of an agent from a loaded red blood cell at a target site by a stimulus, *e.g.*, ultrasound (*see e.g.*, page 3, lines 14-16).

The electrosensitised red blood cells of the invention are distinguishable over the red blood cells loaded with agents by electroporation as generally performed in the art. As an example, Applicants respectfully direct the Examiner's attention to Example 2 of the specification (page 37, line 19 to page 38, line 15). The relative MFI (mean fluorescence

intensity, defined as the ratio of fluorescence associated with loaded cells divided by the fluorescence associated with non-specific binding, *see, e.g.,* page 37, lines 4-7), is 15.2 in non-presensitised cells as compared to an MFI of 61.7 in presensitised cells, demonstrating the dramatic increase in loading as a result of presensitisation (*see, e.g.,* page 38, lines 6-10). Ultrasound sensitivity is about 30% in the absence of a second sensitisation step and about 90-100% of the cells are ultrasound sensitive following a second sensitisation step (*see, e.g.,* page 38, lines 11-15 and page 41, Table 1). Similar results were observed in a tissue mimicking system (*see, e.g.,* Example 7, page 41, line 8 to page 45, line 6).

The electrosensitised red blood cells of the present invention are structurally different that red blood cells electroporated with agents that are not presensitised and electrosensitised because the presensitised and electrosensitised cells are (1) loaded with more agent than non-presensitised cells and (2) hypersensitive to external stimuli such as ultrasound, as compared to non-electrosensitised cells. These variables are quantifiable and thus can be compared to non-electrosensitised cells. As described above, the amount of agent loaded into a cell can be quantifiable by measuring the MFI. Hypersensitivity to disruptive stimuli can be quantified depending on the type of agent loaded into the cell. For example, if an enzyme is the loaded agent, hypersensitivity to disruptive stimuli can be quantified by quantifying the release of enzyme by the cell by assaying enzyme activity (*see e.g.,* page 43, lines 13-26). Alternatively, if oligonucleotides are the loaded agent, hypersensitivity to disruptive stimuli can be quantified by assaying the amount of oligonucleotide release with a spectrofluorimeter (*see e.g.,* page 44, lines 16-26).

Mouneimne relates to CD4 or glycophorin incorporated into a red blood cell. Mouneimne does not teach, suggest or enable an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. At best, Mouneimne relates to electroporated cells, however, such cells are not as ultrasound sensitive as cells that are both presensitised and sensitised (*see e.g.,* page 38, lines 11-15 where sensitised cells are about 30% ultrasound sensitive and presensitised cells and sensitised cells are about 90-100% ultrasound sensitive). Thus, Mouneimne does not teach, suggest or enable the electrosensitised red blood cell of the present invention.

Lizano relates to ADH or ALDH incorporated into a red blood cell. Lizano does not teach, suggest or enable an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. At best, Lizano relates to electroporated cells, however, such cells are not as ultrasound sensitive as cells that are both presensitised and sensitised (*see e.g.,* page 38, lines 11-15 where sensitised cells are about 30% ultrasound sensitive and presensitised cells and sensitised cells are about 90-100% ultrasound sensitive). Thus, Lizano does not teach, suggest or enable the electrosensitised red blood cell of the present invention.

Mitchell relates to rIL2 incorporated into a red blood cell. Mitchell does not teach, suggest or enable an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. At best, Mitchell relates to electroporated cells, however, such cells are not as ultrasound sensitive as cells that are both presensitised and sensitised (*see e.g.,* page 38, lines 11-15 where sensitised cells are about 30% ultrasound sensitive and presensitised cells and sensitised cells are about 90-100% ultrasound sensitive). Thus, Mitchell does not teach, suggest or enable the electrosensitised red blood cell of the present invention.

Zimmermann relates to a medicament or radionuclide incorporated into a red blood cell. Zimmermann does not teach, suggest or enable an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. At best, Zimmermann relates to electroporated cells, however, such cells are not as ultrasound sensitive as cells that are both presensitised and sensitised (*see e.g.,* page 38, lines 11-15 where sensitised cells are about 30% ultrasound sensitive and presensitised cells and sensitised cells are about 90-100% ultrasound sensitive). Thus, Zimmermann does not teach, suggest or enable the electrosensitised red blood cell of the present invention.

Magnani relates to biological agents incorporated into a red blood cell. Magnani does not teach, suggest or enable an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. Unlike the other cited references, Magnani does not even relate to electrosensitised cells, as Magnani introduces agents into red blood cells by concentrating lysed

or partially lysed erythrocyte material before adding biologically active agents to be encapsulated. Thus, Magnani does not teach, suggest or enable the electrosensitised red blood cell of the present invention.

The red blood cells of Mouneimne, Lizano, Mitchell, Zimmerman, or Magnani do not disclose, suggest or enable the electrosensitised red blood cells that are loaded with a higher amount of agent as compared to non-presensitised cells and hypersensitive to a disruptive stimulus as compared to non-electrosensitised cells. Reconsideration and withdrawal of the rejections under 35 U.S.C. § 102(e) are respectfully requested.

5. The Rejections Under 35 U.S.C. § 103 Are Overcome

Claims 1-3, 13-17, 26, 30, 31, 36, 37, 48 and 49 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mitchell in view of Ortiz *et al.*, Mutation Res 1995;327:161-9 (hereinafter "Ortiz"). This rejection is traversed.

Claims 1-4, 13-19, 26, 30, 31 and 36-49 were rejected under 35 U.S.C. § 102(b) as allegedly being unpatentable over Mitchell and Ortiz and further in view of Halaka, U.S. Patent No, 6,071,480 (hereinafter "Halaka"). This rejection is traversed.

The Examiner is respectfully reminded of the case law, namely, that there must be some prior art teaching which would have provided the necessary incentive or motivation for modifying the reference teachings. *In re Laskowski*, 12 U.S.P.Q. 2d 1397, 1399 (Fed. Cir. 1989); *In re Obukowitz*, 27 U.S.P.Q. 2d 1063 (BOPAI 1993). Further, as stated by the Court in *In re Fritch*, 23 U.S.P.Q. 2d 1780, 1783-1784 (Fed. Cir. 1992): "The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification." Also, the Examiner is respectfully reminded that for the § 103 rejection to be proper, both the suggestion of the claimed invention and the expectation of success must be founded in the prior art, and not Applicants' disclosure. *In re Dow*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

Applying the law to the instant facts, the references relied upon by the Office Action does not provide the necessary incentive or motivation for modifying the reference teachings to result in Applicants' invention. At the outset, Applicants respectfully point out that claim 49 has been cancelled, thereby obviating the rejection to claim 49. Claim 1 relates to a method for pre-

sensitizing a red blood cell *in vitro* or *ex vivo* and loading the cell with an agent, wherein the pre-sensitizing step and loading step are temporally separated. Claims 2, 3, 13-19, and 36-48 depend from claim 1. Claim 4 relates to a method for pre-sensitising a red blood cell *in vitro* or *ex vivo*, loading the cell with an agent, electrosensitising the cell *in vitro* or *ex vivo*, and effectuating release of the agent from the cell, wherein the pre-sensitizing step and loading step are temporally separated. As stated above, claims 26 and 30 relate to compositions comprising an electrosensitised red blood cell that is loaded with a higher amount of agent as compared to a non-presensitised cell and hypersensitive to a disruptive stimulus as compared to a non-electrosensitised cell. Claims 31 and 32 depend from claim 30.

Claims 1 and 4 clarify that the first step of presensitising the cell and the second step of loading the cell with an agent are temporally separated, i.e., the two steps are not simultaneous. The electroporation technique of Mitchell requires that red blood cells and rIL-2 are loaded into a polycarbonate chamber and pulsed, i.e., the electric pulse and insertion of rIL-2 is simultaneous. There is no teaching, suggestion or motivation in Mitchell for a first electric pulse to be temporally followed by a second step of loading. Moreover, there is no suggestion in Mitchell to modify the electroporation technique so as to use multiple doses of electroporation. At best, Mitchell describes the use of increased pulse length, increased number of pulses, and increased field intensity to increase hemoglobin release, which is based on the rationale that the creation of electropores large enough to allow hemoglobin release would permit entry of rIL-2.

Similarly, in Ortiz involves the electroporation of a mixture of restriction enzyme and trypsinized CHO cells. There is no teaching, suggestion or motivation in Ortiz for a first electric pulse to be temporally separated by a second step of loading. Contrary to the Examiner's contentions, the first electroporation of Ortiz is not analogous to presensitisation because the first electroporation in Ortiz involves a restriction enzyme, i.e., electroporation and loading are simultaneous.

Halaka does not cure the deficiency of Mitchell or Ortiz by teaching or suggesting a temporal separation between presensitising a cell and loading the cell with an agent. Contrary to the Examiner's contentions, there is no suggestion in Mitchell or Ortiz to choose various power levels for content release of loaded red blood cells. At best, Mitchell describes the use of increased pulse length, increased number of pulses, and increased field intensity to increase

hemoglobin release. Ortiz does not even suggest content release of loaded cells, as Ortiz relates to the insertion of restriction enzymes into CHO cells to study recombination between non-homologous termini produced by the inserted restriction enzymes, i.e., the restriction enzymes remain inside of the CHO cells and are not released.

As stated above, the electrosensitised red blood cells of claim 26 are not merely red blood cells loaded with agents by electroporation as generally performed in the art. Rather, the electrosensitised red blood cells have efficient loading characteristics and are more susceptible to disruption by exposure to a stimulus. Also, as stated above, Mitchell relates to rIL2 incorporated into a red blood cell. Mitchell does not teach, suggest or enable an electrosensitised red blood cell loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. At best, Mitchell relates to electroporated cells, however, such cells are not as ultrasound sensitive as cells that are both presensitised and sensitised (*see e.g.,* page 38, lines 11-15 where sensitised cells are about 30% ultrasound sensitive and presensitised cells and sensitised cells are about 90-100% ultrasound sensitive). Thus, Mitchell does not teach, suggest or enable the electrosensitised red blood cell of the present invention. Moreover, there is no suggestion in Mitchell to modify the electroporation technique so as to use multiple doses of electroporation. At best, Mitchell describes the use of increased pulse length, increased number of pulses, and increased field intensity to increase hemoglobin release, which is based on the rationale that the creation of electropores large enough to allow hemoglobin release would permit entry of rIL-2.

Ortiz does not correct the deficiency of Mitchell by teaching or suggesting sensitised red blood cells that can be loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. Instead, Ortiz relates to double electroporation of cells, which the Examiner believes corresponds to the presensitisation and electrosensitisation steps of the present invention. Applicants respectfully point out that the combination of Mitchell and Ortiz does not result in the electrosensitised cells of the present invention, which are electrosensitised red blood cells loaded with a higher amount of agent and hypersensitive to a disruptive stimulus relative to cells that are not presensitised and electrosensitised. The first step of electroporation in Ortiz is analogous to electroporation as generally performed in the art. The second step of electroporation in Ortiz does not result in

hypersensitivity to a disruptive stimulus, but instead results in the insertion of a second restriction enzyme. The combination of Mitchell and Ortiz does not teach or suggest the electrosensitised red blood cells of the present invention. Moreover, there is no suggestion in Mitchell for the dual electroporation method of Ortiz.

Similarly, Halaka does not cure the deficiencies of Mitchell and Ortiz. Contrary to the Examiner's contentions, there is no suggestion in Mitchell or Ortiz to choose various power levels for content release of loaded red blood cells. At best, Mitchell describes the use of increased pulse length, increased number of pulses, and increased field intensity to increase hemoglobin release. Ortiz does not even suggest content release of loaded cells, as Ortiz relates to the insertion of restriction enzymes into CHO cells to study recombination between non-homologous termini produced by the inserted restriction enzymes, i.e., the restriction enzymes remain inside of the CHO cells and are not released.

It would not have been obvious to one of ordinary skill in the art, at the time the invention was made, to modify the methods taught by Mitchell and Ortiz to use the protocol of multiple doses of electroporation for loading multiple agents into red blood cells with a reasonable expectation of successs. Contrary to the Examiner's contention, there is not teaching or suggestion in Mitchell or Ortiz that one of ordinary skill of the art would be motivated to combine the references for separate loading of agents or for easier release at a later time with multiple electroporation steps. Even assuming, *arguendo*, that there was motivation to combine the references, the combination of Mitchell and Ortiz does not result in the claimed method of producing a red blood cell loaded with an agent, a method for releasing an agent from such a cell, or the presensitised red blood cells of the invention.

Similarly, it would not have been obvious to one of ordinary skill in the art, at the time the invention was made, to modify the methods taught by Mitchell, Ortiz, and Halaka by choosing various power levels for content release of loaded red blood cells with a reasonable expectation of success. Contrary to the Examiner's assertion, there is not teaching or suggestion in Mitchell, Ortiz or Halaka that one of ordinary skill of the art would be motivated to combine the references to optimize the energy power according to the cells and loading agents. Even assuming, *arguendo*, that there was motivation to combine the references, the combination of Mitchell, Ortiz and Halaka does not result in the claimed method of producing a red blood cell

loaded with an agent, a method for releasing an agent from such a cell, or the presensitised red blood cells of the invention.

Reconsideration and withdrawal of the rejections under 35 U.S.C. § 103(a) are respectfully requested.

6. The Double Patenting Rejections Are Overcome

Claims 1, 3, 10, 13-17, 26, 30, 31, 36, 37, 48 and 49 were rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 19-21 of U.S. Application No. 09/779,186, now U.S. Patent No. 6,495,351.

Claims 4, 18, 19, 36, 37, 48 and 49 were rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 8-14 of co-pending U.S. Application No. 09/748,063.

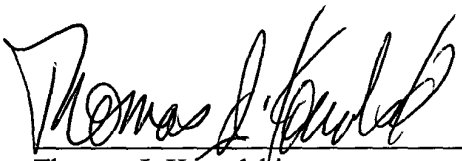
The issue of whether there is indeed double patenting is contingent upon whether new claims herewith are indeed considered and entered; and, if so, whether the Examiner believes there is overlap with claims ultimately allowed in the co-pending application. If upon agreement as to allowable subject matter it is believed that there is still a double patenting issue, then, at that time, if indeed necessary, a Terminal Disclaimer as to U.S. Application No. 09/779,186, now U.S. Patent No. 6,495,351 and co-pending U.S. Application No. 09/748,063 will be filed.

Accordingly, reconsideration and withdrawal of the double patenting rejection, or at least holding it in abeyance until agreement is reached as to allowable subject matter, is respectfully requested.

CONCLUSION

In view of the remarks and amendments herewith, the application is believed to be in condition for allowance. Favorable reconsideration of the application and prompt issuance of a Notice of Allowance are earnestly solicited. The undersigned looks forward to hearing favorably from the Examiner at an early date, and, the Examiner is invited to telephonically contact the undersigned to advance prosecution. The Commission is authorized to charge any fee occasioned by this paper, or credit any overpayment of such fees, to Deposit Account No. 50-0320.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: 

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